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(54) Title: USES OF 1,25-DIHYDROXY-5,6-*trans* VITAMIN D COMPOUNDS AND DERIVATIVES THEREOF

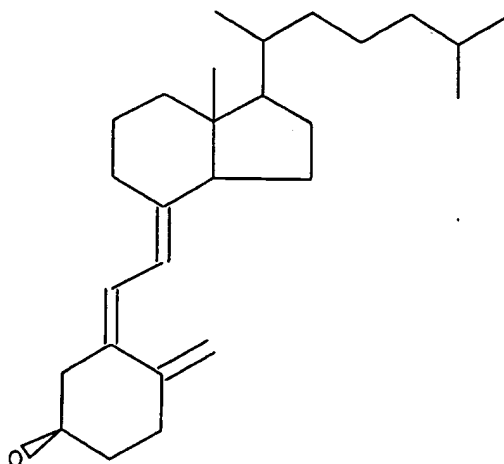
(57) Abstract: 1,25-dihydroxy-5,6-*trans* vitamin D compounds and their derivatives, especially the esters, are useful in the treatment or prevention of diseases characterised by abnormal cell differentiation or cell proliferation, as well as other diseases or conditions treatable with vitamin D compounds, such as osteoporosis and other metabolic bone diseases, and are not associated with the degree of hypercalcaemia observed with 1,25-dihydroxy vitamin D₃.

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USES OF 1,25-DIHYDROXY-5,6-*trans* VITAMIN D COMPOUNDS
AND DERIVATIVES THEREOF

The present invention relates to the use of vitamin D analogues and their derivatives in medicine, particularly in the treatment or prevention of diseases characterised by abnormal cell differentiation or cell proliferation, as well as other diseases or conditions treatable with vitamin D compounds, including renal osteodystrophy, hypoparathyroidism, and metabolic bone diseases such as osteoporosis.

Vitamin D₃ has the structure:



It is well known that vitamin D₃ plays a vital role in the metabolism of calcium, by promoting intestinal absorption of calcium and phosphorus, maintaining adequate serum levels of calcium and phosphorus, and stimulating mobilisation of calcium from the bone fluid compartment in the presence of parathyroid hormone [Holick, M. F., in "Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism", M. J. Favus (ed.), 4th Ed., Lippincott-Williams & Wilkins: Philadelphia (1999), pp. 92-98].

To become biologically active, vitamin D₃ must undergo two hydroxylation steps. The first hydroxylation step occurs in the liver, by hydroxylation at C-25, thereby forming 25-hydroxyvitamin D₃ [25-(OH)D₃], the major circulating metabolite of vitamin D₃. The second hydroxylation occurs in the kidney, by hydroxylation at the 1 α -position, forming 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂-D₃], which is the hormonally active metabolite.

1,25-(OH)₂-D₃ is the most active form of vitamin D, and is intimately involved in calcium and phosphorous homeostasis, both in animals and humans. Its principal physiological function is on calcium and bone metabolism, stimulating intestinal calcium transport and initiating mobilisation of calcium stores from bone, as noted above. Patients with kidney failure, so who are unable to make 1,25-(OH)₂-D₃, develop hypocalcaemia, secondary hyperparathyroidism, and the bone disease renal osteodystrophy. Accordingly, administration of 1,25-(OH)₂-D₃ is useful in the treatment of disorders of calcium and bone metabolism, such as renal osteodystrophy, vitamin D-dependent rickets, low blood serum calcium due to hypoparathyroidism, and particularly osteoporosis, as it compensates for the lack of the naturally occurring metabolite, and stimulates calcium uptake in the gut.

1,25-(OH)₂-D₃ is able to carry out most of its biological effects by interacting with a specific receptor known as the vitamin D receptor (VDR). VDR is present in tissues that are responsible for regulating calcium and bone metabolism, including the small intestine, kidney and bone. In addition, a wide variety of tissues and cells, not related to calcium and bone metabolism, also possess VDR (Holick, M. F., "Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism", *supra*). These include, among others, brain, heart, gonads, breast, pancreas, skeletal muscle, smooth muscle, skin, monocytes, and activated T and B lymphocytes. In addition, several tumour cell lines, including promyelocytic leukaemic cells, multiple myeloma cells, squamous cell carcinoma cells, prostate cancer cells, breast cancer cells, and basal cell carcinoma cells, possess VDR [Holick, M. F., Bone, 17, (Suppl.):107S-111S (1995)].

Although the exact function of VDR in such tissues and cells is not well understood, it is known that some cells possessing VDR decrease their proliferative activity in response to $1,25-(\text{OH})_2\text{-D}_3$. Multiple studies have shown that several lines of cancer cells possessing a VDR, including colon, melanoma, prostate and breast, when incubated with $1,25-(\text{OH})_2\text{-D}_3$, decreased their proliferation [Holick, M. F., Bone, *supra*; Halline, A. G., *et al.*, *Endocrinology* 134:1710-1717 (1994); Konety, B. R., *et al.*, *Cell Growth & Differentiation* 7:1563-1570 (1996); and Kivineva, M., *et al.*, *J. Steroid Biochem. Mol. Biol.* 66:121-127 (1998)]. In addition, some cells, such as keratinocytes, are also induced into terminal differentiation.

Thus, $1,25-(\text{OH})_2\text{-D}_3$ has been shown to stimulate the differentiation of cells and to inhibit excessive cell proliferation [*c.f.* Walter, M. R., *Endocr. Rev.* 13:719-764 (1992); see also Schwartz, G. G., *Anticancer Res.* 14:1077-1082 (1994); Amento *et al.*, *J. Clin. Invest.* 73:731-739 (1984); Tanaka *et al.*, *Biochem. J.* 204:713-719 (1982); Abe, E., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.* 78:4990-4994 (1981); and Colston *et al.*, *Endocrinology* 108:1083-1086 (1981)]. This effect has given rise to the use of $1,25-(\text{OH})_2\text{-D}_3$ and its analogues for treating hyperproliferative skin diseases such as psoriasis, and has suggested the possibility for treating some cancers such as breast, colon, and prostate [Holick, M. F., Bone *supra*; Gross, C., *et al.*, in *Vitamin D*, D. Feldman *et al.* (eds.), Academic Press: New York (1997), pp. 1125-1139; U.S. Patent Nos. 4,728,643 and 5,037,816].

In addition to regulating calcium and phosphorus homeostasis and stimulating cell growth and differentiation, $1,25-(\text{OH})_2\text{-D}_3$ has also been shown to have various additional effects on cellular metabolism.

For example, a potential therapeutic use for $1,25-(\text{OH})_2\text{-D}_3$ is suggested by the observation of an association between hereditary vitamin D resistance and alopecia: treatment with $1,25-(\text{OH})_2\text{-D}_3$ may promote hair growth [Editorial, *Lancet*, Mar. 4, (1989), p. 478]. Moreover, the fact that topical application of $1,25-(\text{OH})_2\text{-D}_3$ reduces the size of sebaceous glands in the ears of male Syrian hamsters suggests that this

compound might be useful for the treatment of acne [Malloy, V. L., *et al.*, The Tricontinental Meeting for Investigative Dermatology, Washington, (1989)]. Use of 1,25-(OH)₂-D₃, or its pro-drug, 1-(OH)D₃, for the treatment of hypertension [Lind, L., *et al.*, *Acta Med. Scand.* 222:423-427 (1987)] and diabetes mellitus [Inomata, S., *et al.*, *Bone Mineral.* 1:187-192 (1986)], has also been suggested.

The potential therapeutic applications suggested above, however, are severely limited by the risk of hypercalcaemia, a condition resulting from elevated blood serum concentrations of 1,25-(OH)₂-D₃ which often occurs during exogenous administration of the compound (Holick, M. F., "Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism", *supra*). Hypercalcaemia is a potentially fatal condition, if not controlled, making administration of large amounts of 1,25-(OH)₂-D₃ undesirable. Further, even topical administration has been demonstrated to lead to elevated levels of serum calcium, so that administration of 1,25-(OH)₂-D₃ is, therefore, not even completely satisfactory for use as a drug in the treatment of, for example, psoriasis or leukaemia, which may require continuous administration of the drug in relatively high doses.

Accordingly, a number of structural analogues of 1,25-(OH)₂-D₃ and its precursors have been prepared, including fluorinated analogues [*c.f.* DeLuca and Schnoes, *Ann. Rev. Biochem.* 52:411-439 (1983)] and compounds containing the vitamin D nucleus, but with altered or additional side chains (*c.f.* U.S. Patent Nos. 5,945,410; 5,932,565; 5,843,928; and 5,554,599).

The *trans* forms of both vitamin D₃ and 25-hydroxyvitamin D₃ [5,6-*trans* D₃ and 5,6-*trans*-25-(OH)D₃] have also been prepared as vitamin D₃ structural analogues [Holick *et al.*, *Biochemistry* 11:2715 (1972); Holick *et al.*, *Science* 176:1247 (1972)]. These latter two compounds possess biological activity similar to that of naturally occurring 1,25-(OH)₂-D₃, in that each stimulates a biological response in anephric animals similar to that elicited by 1,25-(OH)₂-D₃. 5,6-*trans* D₃ stimulates intestinal calcium transport as well as bone calcium mobilisation from bone in anephric rats,

while 5,6-*trans*-25-(OH)D₃ only stimulates intestinal calcium transport [Holick *et al.*, Biochemistry 11:2715 (1972); Holick *et al.*, Science 176:1247 (1972)]. The naturally occurring *cis* analogues, D₃ and 25-(OH)D₃, are inactive in anephric rats.

A need continues to exist for new methods for the treatment of disorders involving calcium and phosphorus metabolism, such as osteoporosis, and the treatment or prevention of diseases characterised by abnormal cell differentiation or cell proliferation, as well as other diseases or conditions treatable with vitamin D compounds.

Surprisingly, the *trans* forms of the 1,25-dihydroxyvitamin D compounds, while retaining many, if not all, of the therapeutic advantages of the naturally occurring *cis* compounds, are significantly less active in the gut, thereby allowing use of greater amounts of the *trans* form, without the risk of hypercalcaemia associated with the naturally occurring *cis* compounds.

Thus, in a first aspect, the present invention provides a 1,25-dihydroxy-5,6-*trans* vitamin D compound, or a pharmaceutically acceptable derivative thereof, for use in therapy.

The present invention extends to any vitamin D compound, but applies, more preferably to vitamins D₂ and D₃. Of these, vitamin D₃ is particularly preferred, with vitamin D₂ generally being used as a vitamin D₃ substitute. Thus, the preferred compound for use in the present invention is 1,25-dihydroxy-5,6-*trans* vitamin D₃. Where vitamin D₃ and its hydroxy, and *trans* analogues, is mentioned herein, then it will be understood that reference is also had to other vitamin D compounds, unless otherwise indicated or apparent.

By pharmaceutically acceptable derivative is meant anything that can be administered to a patient without unacceptable levels of toxicity. In particular, there are provided the esters and orthoesters of vitamin D, especially vitamin D₃. Another

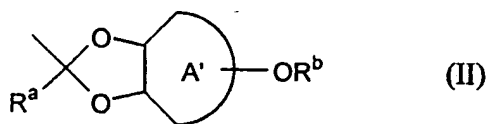
preferred group of derivatives is the glycosides and their amino derivatives.

When the derivative of the present invention is a glycoside or orthoester glycoside derivative, then it may be straight or branched chain, and may be a mono- or di- glycoside, that is, it occurs on either the 1- or 25- positions, or both. In addition, the derivative may be mixed with, for example, a glycoside in the 1- position and a citrate in the 25- position, although there will generally be no requirement for such a compound which, in any case, is generally more difficult to prepare. Thus, as with other derivatives, the diglycoside is generally preferred, for ease of manufacture.

It will also be appreciated that the glycosides, as with other hydroxyester side chains, may be further esterified, preferably by lower alkanoyl groups, especially acetyl groups.

Preferred glycosides contain 1-20 glycosidic units. More preferably, the glycoside is a mono-, di- or tri- saccharide, and is preferably a glycopyranosyl group, particularly a 1'- β -glucopyranosyl group.

When the derivative is an orthoester glycoside, then it preferably has the Formula (II):



wherein A' represents a glycofuranosyl or glycopyranosyl ring;

R_a is H, C₁₋₄ alkyl, C₇₋₁₀ aralkyl, phenyl or phenyl substituted by chloro, fluoro, bromo, iodo, C₁₋₄ alkyl or C₁₋₄ alkoxy, or is naphthyl; and

R_b is H or a straight or branched chain glycosidic residue containing 1-20 glycosidic units.

In general, the derivatives of the present invention serve as protected precursors of the parent vitamin D compound, and the protecting group is metabolically cleaved to yield the active compound.

Essentially, 1,25-dihydroxy-5,6-*trans* vitamin D₃ and its esters can be used, instead of 1,25-dihydroxyvitamin D₃, wherever the latter would otherwise be indicated, save where increased calcium uptake from the gut is specifically required. The compounds of the invention also have the advantage that they do not stimulate loss of calcium from bones and, may be used, if deemed appropriate by a skilled clinician, together with 1,25-(OH)₂vitamin D₃, if desired.

In general, it is preferred to use the unesterified 1,25-dihydroxy-5,6-*trans* vitamin D₃, whether for oral or topical administration. Although other administration forms are not ruled out, the most convenient routes of administration for any form of vitamin D are oral and topical.

Any pharmaceutically acceptable ester may be employed, provided that, *in situ*, 1,25-dihydroxy-5,6-*trans* vitamin D₃ will be available. Thus, for topical administration, as there will be little or no metabolic processing prior to reaching the target cells, the preferred form is the parent compound.

However, for oral administration, in order to reduce the effect on mucosal uptake of calcium, it may be preferred to employ an ester. Suitable esters include, for example, the acetate, citrate and glucuronic esters.

The present invention further provides a medicament, or pharmaceutical composition, comprising 1,25-dihydroxy-5,6-*trans* vitamin D₃, or a pharmaceutically acceptable ester thereof, and a pharmaceutically acceptable carrier therefor.

The invention further provides a method for the treatment or prophylaxis of a condition, other than hypocalcaemia, responsive to treatment with a vitamin D

compound, especially 1,25-dihydroxyvitamin D₃, comprising administering 1,25-dihydroxy-5,6-*trans* vitamin D₃, or a pharmaceutically acceptable ester thereof, to an animal in need thereof. The animal is preferably mammalian and, more preferably, human.

In the alternative, there is provided a method for the treatment or prophylaxis of a disease or condition characterised by abnormal cell differentiation or cell proliferation in an animal, comprising administering to the cells of the animal in need thereof an effective amount of 1,25-dihydroxy-5,6-*trans* vitamin D₃, or a pharmaceutically acceptable ester thereof, whereby the disease or condition is treated or prevented.

It will be appreciated that there is also provided use of 1,25-dihydroxy-5,6-*trans* vitamin D₃, or a pharmaceutically acceptable ester thereof, in the manufacture of a medicament for the treatment or prophylaxis of a disease or condition as described herein, particularly those treatable by 1,25-dihydroxy vitamin D₃, other than hypocalcaemia.

The present invention also extends to the novel derivatives, especially the esters, of 1,25-dihydroxy-5,6-*trans* vitamin D₃ and D₂, especially the acetate, citrate and glucuronic esters of vitamin D₃, the acetate being most preferred. The esters may be single or double esters, the double esters generally being preferred.

A suitable category of condition for treatment with compounds of the present invention include metabolic bone diseases (including osteoporosis, for example), renal osteodystrophy, and hypoparathyroidism.

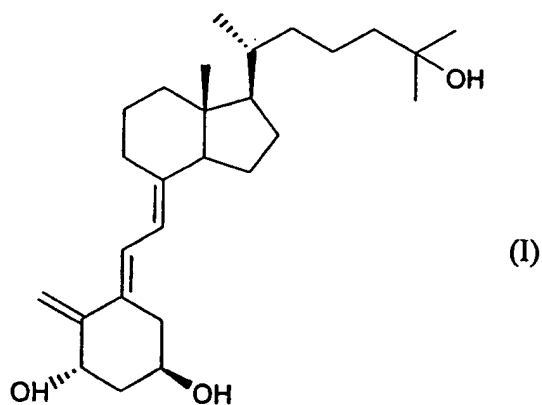
Another suitable category is those diseases and conditions characterised by abnormal cell differentiation or cell proliferation. In particular, hyperproliferative skin disorders may be targetted, and these include psoriasis, ichthyosis, and actinic keratoses.

Another preferred disease category for treatment or prophylaxis, including after care, is cancer. Compounds of the invention may be used to inhibit the proliferation of tumour cells, or used to induce differentiation of tumour cells. Suitable targets include prostate cancer, breast cancer, skin cancer, colon cancer, leukaemias, lymphomas, and lung cancer.

Disorders in calcium and bone metabolism may also be treated. Suitable disorders for treatment include; vitamin D-dependent rickets type I, X-linked hypophosphataemic rickets, vitamin D-dependent rickets type II, renal osteodystrophy, low blood serum calcium due to hypoparathyroidism, and, in particular, osteoporosis.

Figure 1 shows the effect of 5,6-*trans*-1,25-(OH)₂-D₃ in comparison to 1,25-(OH)₂-D₃ on inhibition of proliferation of cultured human keratinocytes.

1,25-dihydroxy-5,6-*trans* vitamin D₃ [5,6-*trans*-1,25-(OH)₂-D₃] has the Formula (I):



Hyperproliferative skin disorders include such disorders as psoriasis, ichthyosis, and actinic keratoses, a pre-skin cancer, and even such conditions as benign prostatic hyperplasia. Administration of 5,6-*trans*-1,25-(OH)₂-D₃ results in a

decrease in proliferative activity and induces the affected cells to differentiate into normal morphology.

Preferred cancers to be targetted by the present invention are those whose cells express the vitamin D receptor (VDR), including prostate cancer, breast cancer, skin cancer, colon cancer, leukemias, lymphomas, and lung cancer.

Disorders in calcium and bone metabolism include such conditions as renal osteodystrophy, vitamin D-dependent rickets type I, X-linked hypophosphataemic rickets, vitamin D-dependent rickets type II, and osteoporosis, as well as such disorders in calcium and bone metabolism as low blood serum calcium due to hypoparathyroidism. It is particularly preferred to treat osteoporosis.

5,6-*trans*-1,25-(OH)₂-D₃ can be synthesised following any known method of synthesis of the compound, such as that described in Andrews, D. R., *et al.*, J. Org. Chem. 51(25):4819-4828 (1986), which relates to the synthesis of 1,25-(OH)₂-D₃.

The compounds of the present invention are intended for use in pharmaceutical compositions which are useful in the treatment of human and veterinary disorders as described above.

5,6-*trans*-1,25-(OH)₂-D₃ can be administered in any appropriate pharmaceutically acceptable carrier for oral, parenteral, or topical administration. It can be administered by any suitable means for the treatment or prevention of conditions or diseases as exemplified herein, including diseases characterised by abnormal cell differentiation or cell proliferation, or other conditions treatable with vitamin D compounds (metabolic bone diseases such as osteoporosis, or inflammation, for example), in animals, especially humans.

Suitable mammals for treatment include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits, mice and humans. Particularly preferred are humans.

Dosage forms of the various compounds can be prepared by combining them with non-toxic pharmaceutically acceptable carriers to make either immediate release or slow release formulations, as is well known in the art. Such carriers may be either solid or liquid such as, for example, corn starch, lactose, sucrose, peanut oil, olive oil, sesame oil and propylene glycol. If a solid carrier is used the dosage form of the compounds may be tablets, capsules, powders, troches or lozenges. If a liquid carrier is used, soft gelatin capsules, or syrup or liquid suspensions, emulsions or solutions may be the dosage form. The dosage forms may also contain adjuvants, such as preserving, stabilising, wetting or emulsifying agents, solution promoters, *etc.* They may also contain other therapeutically active agents.

The dosage administered will be an amount effective to achieve the intended purpose and will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. For example, systemic daily dosage of 5,6-*trans*-1,25-(OH)₂-D₃ will be from about 0.001 micrograms/kg to 100 micrograms/kg, preferably 0.01 to 10.0 micrograms per kg of body weight. Normally, from about 0.1 to 100 micrograms/kg per day of 5,6-*trans*-1,25-(OH)₂-D₃, in one or more dosages per day, is effective to obtain the desired results. Suitable topical dosages are from 0.001 micrograms to 100 micrograms/cm² area of skin. One of ordinary skill in the art can determine the optimal dosage and concentration with only routine experimentation.

5,6-*trans*-1,25-(OH)₂-D₃ can be employed in dosage forms such as tablets, capsules, powder packets, or liquid solutions, suspensions or elixirs for oral administration, sterile liquid for formulations, such as solutions or suspensions for parenteral use. Alternatively, the compound may be administered transdermally *via* a patch or ointment and the like. 5,6-*trans*-1,25-(OH)₂-D₃, as the active ingredient, will ordinarily be present in an amount of at least 10⁻⁶ % by weight based upon the total weight of the composition, and not more than 90% by weight. An inert pharmaceutically acceptable carrier is preferably used. Among such carriers include 95% ethanol, vegetable oils, propylene glycols, saline buffers, *etc.*

Formulations suitable for topical administration include liquid or semi-liquid preparations such as liniments, lotions, applicants, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes; or solutions or suspensions such as drops; or as sprays.

The topical compositions of this invention are formulated preferably as creams, lotions, ointments and the like by choice of appropriate carriers. Suitable carriers include vegetable or mineral oils, white petrolatum (white soft paraffin), branched chain fats or oils, animal fats and high molecular weight alcohol (greater than C₁₂). The preferred carriers are those in which the active ingredient is soluble. Emulsifiers, stabilisers and antioxidants may also be included as well as agents imparting colour or fragrance if desired.

Topical creams are preferably formulated from a mixture of mineral oil, self-emulsifying beeswax and water in which mixture the active ingredient, dissolved in a small amount of an oil such as almond oil, is admixed. A typical example of such a cream is one which includes about 39 parts water, about 20 parts beeswax, about 40 parts mineral oil and about 1 part almond oil.

Topical ointments may be formulated by mixing a solution of the active ingredient in a vegetable oil such as almond oil with warm soft paraffin and allowing the mixture to cool. A typical example of such an ointment is one which includes about 30% almond oil and about 70% white soft paraffin by weight.

Topical lotions may be conveniently prepared by dissolving the active ingredient, in a suitable high molecular weight alcohol such as propylene glycol or polyethylene glycol.

The following Examples are illustrative, but not limiting, of the methods of the present invention.

EXAMPLE 1

PREPARATION OF 1,25-DIHYDROXY-5,6-TRANS VITAMIN D₃

5,6-*trans*-1,25-(OH)₂-D₃ can be synthesised following any known method of synthesis of the compound, such as that described in Andrews, D. R., *et al.*, J. Org. Chem. 51(25):4819-4828 (1986). Following this technique, crystals of the title compound, were analysed using standard protocols, and had the following properties:

Melting point: 172-173°C;

Optical rotation: $[\alpha]_D +189.09 \text{ @ } 0.165, \text{ EtOH}$;

Absorption spectrum: UV (EtOH): 208 nm (E13,380), 273 (24,100);

Analysis: Calculated: C 77.84, H 10.65. Found: C 77.63, H 10.61;

¹NMR (CD₃OD): δ 0.60 (s, CH₃, 3H), 0.98 (d, CH-CH₃, 3H), 1.19 (s, CH₃, 3H), 2.60-3.00 (m, =CH + CH₂, 3H), 4.09 (m, CH-OH, 1H), 4.40 (m, CH-OH, 1H), 4.95 and 5.05 (d, =CH₂, 2H), 5.89 (d, =CH, 1H), 6.52 (d, =CH, 1H).

EXAMPLE 2

EFFECT OF 5,6-TRANS-1,25-(OH)₂-D₃ ON INHIBITION OF PROLIFERATION OF CULTURED HUMAN KERATINOCYTES

The effect of 5,6-*trans*-1,25-(OH)₂-D₃ on inhibition of the proliferation of cultured human keratinocytes was tested and compared to that of 1,25-(OH)₂-D₃.

Second passage normal human keratinocytes were treated with 0, 10⁻⁹, 10⁻⁸, and 10⁻⁷ M of 1,25-(OH)₂-D₃ or 5,6-*trans*-1,25-(OH)₂-D₃ in the presence of basal MCDB media plus 25 ng/ml EGF at 37°C, following experimental methods previously described [Smith, E. L., Walworth, N., Holick, M.F., "Effect of 1,25-

dihydroxy vitamin D₃ on the morphologic and biochemical differentiation of cultured human epithelial keratinocytes grown in serum-free conditions," J. Invest. Dermatol. 86:709-714 (1986)]. Eighteen hours later, [³H]-thymidine incorporation into DNA was performed.

The results, expressed as percent of control, are shown in Figure 1 [Key - Cultured human keratinocytes were incubated with 5,6-*trans*-1,25-(OH)₂-D₃ or 1,25-(OH)₂-D₃ at different concentrations (1nM, 10nM, 100nM) for eighteen hours. [³H]thymidine incorporation was measured as described. •-•: [³H]thymidine incorporation of cells incubated with 1,25-(OH)₂-D₃; □-□: [³H]thymidine incorporation of cells incubated with 5,6-*trans*-1,25-(OH)₂-D₃. Results are expressed as percent of control values].

As Figure 1 indicates, 5,6-*trans*-1,25-(OH)₂-D₃ inhibits the growth of normal human keratinocytes, and it does so to roughly the same degree as its naturally-occurring *cis* analogue, 1,25-(OH)₂-D₃.

Having now fully described the invention, it will be understood to those of ordinary skill in the art that the same can be performed within a wide and equivalent range of conditions, formulations, and other parameters without affecting the scope of the invention or any embodiment thereof. All patents and publications cited herein are fully incorporated by reference herein in the entirety.

Claims:

1. A 1,25-dihydroxy-5,6-*trans* vitamin D compound, or a pharmaceutically acceptable derivative thereof, for use in therapy.
2. A compound for use according to claim 1, which is 1,25-dihydroxy-5,6-*trans* vitamin D₃, or a pharmaceutically acceptable derivative thereof, for use in therapy.
3. A compound for use according to claim 1, which is 1,25-dihydroxy-5,6-*trans* vitamin D₂, or a pharmaceutically acceptable derivative thereof, for use in therapy.
4. A compound for use according to any preceding claim, which is a glycoside or orthoester glycoside derivative of said vitamin D compound.
5. A compound as defined in of claims 1 to 3, in the form of the acetic, citric or glucuronic ester thereof.
6. A compound according to any preceding claim, for use in an indication in which 1,25-dihydroxyvitamin D₃ would otherwise be indicated.
7. A compound according to any preceding claim, for use in the treatment or prophylaxis of a disease or condition characterised by abnormal cell differentiation or cell proliferation.
8. A compound for use according to claim 7, wherein the disease or condition is a hyperproliferative skin disorder.
9. A compound for use according to claim 8, wherein the hyperproliferative skin disorder is selected from psoriasis, ichthyosis, and actinic keratoses.
10. A compound for use according to claim 7, wherein the disease or condition is

prostate cancer, breast cancer, skin cancer, colon cancer, lung cancer, leukaemia, or lymphoma.

11. A compound for use according to claim 7, wherein the disease or condition is a disorder in calcium and bone metabolism.

12. A compound for use according to claim 11, wherein the disorder in calcium and bone metabolism is vitamin D-dependent rickets type I, X-linked hypophosphataemic rickets, vitamin D-dependent rickets type II, or osteoporosis.

13. A compound for use according to claim 11, wherein the disorder is renal osteodystrophy.

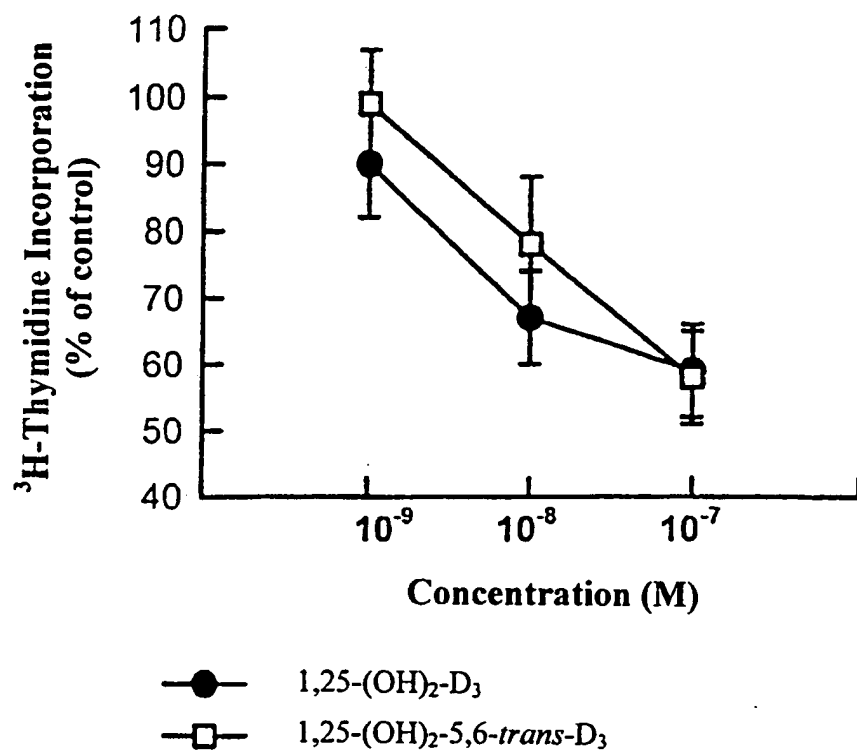
14. A compound for use according to claim 11, wherein the disorder is low blood serum calcium due to hypoparathyroidism.

15. A compound for use according to claim 11, wherein the disorder is osteoporosis.

16. A compound according to any preceding claim, in a formulation suitable for oral administration.

17. A compound according to any of claims 1 to 15, in a formulation suitable for topical administration.

18. A compound according to any of claims 1 to 15, in a formulation suitable for systemic administration.

**Fig. 1**

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(54) Title: **USES OF 1,25-DIHYDROXY-5,6-*trans* VITAMIN D COMPOUNDS AND DERIVATIVES THEREOF**

(57) Abstract: 1,25-dihydroxy-5,6-*trans* vitamin D compounds and their derivatives, especially the esters, are useful in the treatment or prevention of diseases characterised by abnormal cell differentiation or cell proliferation, as well as other diseases or conditions treatable with vitamin D compounds, such as osteoporosis and other metabolic bone diseases, and are not associated with the degree of hypercalcaemia observed with 1,25-dihydroxy vitamin D₃.



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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93 09093 A (RESEARCH INSTITUTE FOR MEDICINE AND CHEMISTRY) 13 May 1993 (1993-05-13) page 5, line 14 -page 9, line 30 ---	1-18
Y	US 5 932 565 A (GUNNAR GRUE-SORENSEN) 3 August 1999 (1999-08-03) cited in the application column 1 -column 3, line 45 ---	1-18
Y	US 5 565 589 A (HECTOR F. DELUCA) 15 October 1996 (1996-10-15) column 3 -column 6, line 5 ---	1-18
Y	US 5 554 599 A (GUNNAR GRUE-SORENSEN ET AL.) 10 September 1996 (1996-09-10) cited in the application column 1 -column 3, line 60 ---	1-18
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Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 728 643 A (MICHAEL F. HOLICK ET AL.) 1 March 1988 (1988-03-01) cited in the application the whole document ----	1-18
Y	PATENT ABSTRACTS OF JAPAN vol. 110, no. 24 (C-399), 23 January 1987 (1987-01-23) & JP 61 197573 A (CHUGAI PHARMACEUT CO LTD), 1 September 1986 (1986-09-01) abstract ----	1-18
Y	DATABASE WPI Week 198204 Derwent Publications Ltd., London, GB; AN 1982-06749E XP002184307 "Trans vitamin- D deriv.-specifically (24R)-24,25-Di:hydroxy-5,6-trans vitamin D3" & JP 56 161368 A (CHUGAI PHARM CO LTD), 11 December 1981 (1981-12-11) abstract ----	1-18
Y	DATABASE WPI Week 8516 Derwent Publications Ltd., London, GB; AN 1985-096635 XP002184308 "Pharmaceutical 1 alpha-hydroxy vitamin-D3 cpds.-used for treating arterial sclerosis platelet aggregation thrombos inflammation etc." & JP 60 045516 A (KUREHA CHEM IND CO LTD), 12 March 1985 (1985-03-12) abstract ----	1-18
Y	ANDREW S. CRAIG ET AL.: "Two Novel Allenic Side Chain Analogues of 1 alpha, 25- Dihydroxyvitamin D3" J. ORG. CHEM., vol. 57, no. 16, 1992, pages 4374-4380, XP002086123 columbus ohio the whole document ----	1-18
Y	BRUNO FIGADÈRE ET AL.: "Arocalciferols: Synthesis and Biological Evaluation of Aromatic Side-chain Analogues of 1alpha,25-Dihydroxyvitamin D3" JOURNAL OF MEDICINAL CHEMISTRY, vol. 34, no. 8, 1991, pages 2452-2463, XP002101748 columbus ohio the whole document -----	1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/04120

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9309093	A	13-05-1993	AT 134366 T	15-03-1996
			AU 664213 B2	09-11-1995
			AU 2901492 A	07-06-1993
			CA 2121678 A1	13-05-1993
			CZ 9401112 A3	15-03-1995
			DE 69208478 D1	28-03-1996
			DK 614455 T3	18-03-1996
			WO 9309093 A1	13-05-1993
			EP 0614455 A1	14-09-1994
			ES 2083771 T3	16-04-1996
			FI 942114 A	06-05-1994
			GR 3019601 T3	31-07-1996
			HU 67140 A2	28-02-1995
			JP 7502499 T	16-03-1995
			KR 271461 B1	15-11-2000
			NO 941683 A	27-06-1994
			NZ 245041 A	28-03-1995
			PL 171580 B1	30-05-1997
			RU 2139276 C1	10-10-1999
			SK 52294 A3	09-11-1994
			US 5494905 A	27-02-1996
			US 5686435 A	11-11-1997
			ZA 9208572 A	29-06-1993
US 5932565	A	03-08-1999	AT 206394 T	15-10-2001
			AU 713630 B2	09-12-1999
			AU 2567097 A	29-10-1997
			DE 69707121 D1	08-11-2001
			EP 0891326 A1	20-01-1999
			JP 2000508635 T	11-07-2000
			PL 324635 A1	08-06-1998
			CN 1196720 A	21-10-1998
			CZ 9800008 A3	17-06-1998
			WO 9737972 A1	16-10-1997
			HU 9901600 A2	28-09-1999
US 5565589	A	15-10-1996	AT 175194 T	15-01-1999
			AU 8051594 A	23-05-1995
			DE 69415690 D1	11-02-1999
			DE 69415690 T2	20-05-1999
			DK 726892 T3	30-08-1999
			EP 0726892 A1	21-08-1996
			ES 2126157 T3	16-03-1999
			GR 3029449 T3	28-05-1999
			JP 9504549 T	06-05-1997
			WO 9512575 A1	11-05-1995
			US 5488044 A	30-01-1996
			US 5536828 A	16-07-1996
			US 5484781 A	16-01-1996
			US 5484782 A	16-01-1996
US 5554599	A	10-09-1996	AU 668638 B2	09-05-1996
			AU 5808794 A	19-07-1994
			DE 69307929 D1	13-03-1997
			DE 69307929 T2	04-09-1997
			EP 0675878 A1	11-10-1995
			FI 952972 A	16-06-1995
			GR 3022774 T3	30-06-1997

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/04120

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5554599	A	JP 8504775 T RU 2136660 C1 AT 148454 T CA 2146775 A1 WO 9414766 A1 DK 675878 T3 ES 2098123 T3 NZ 259317 A	21-05-1996 10-09-1999 15-02-1997 07-07-1994 07-07-1994 28-04-1997 16-04-1997 26-05-1997
US 4728643	A	01-03-1988 AT 66780 T DE 3584013 D1 EP 0202276 A1 JP 2664667 B2 JP 62501073 T WO 8602527 A1 US 5037816 A	15-09-1991 10-10-1991 26-11-1986 15-10-1997 30-04-1987 09-05-1986 06-08-1991
JP 61197573	A	01-09-1986 NONE	
JP 56161368	A	11-12-1981 NONE	
JP 60045516	A	12-03-1985 JP 1654708 C JP 3019207 B JP 1795548 C JP 3169817 A JP 5004368 B JP 1763753 C JP 3169820 A JP 4057648 B JP 1795549 C JP 3169818 A JP 5004367 B JP 3169819 A	13-04-1992 14-03-1991 28-10-1993 23-07-1991 19-01-1993 28-05-1993 23-07-1991 14-09-1992 28-10-1993 23-07-1991 19-01-1993 23-07-1991